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Abstract: OBJECTIVE The aim of this study was to define the influence of trauma on cardiac glucose and fatty acid transport. The effects were investigated in vivo in a porcine mono- and polytrauma model and in vitro in human cardiomyocytes, which were treated simultaneously with different inflammatory substances, mimicking post-traumatic inflammatory conditions. METHODS AND RESULTS In the porcine fracture- and polytrauma model, blood glucose concentrations were measured by blood gas analysis during an observation period of 72 h. The expression of cardiac glucose and fatty acid transporters in the left ventricle was determined by RT-qPCR and immunofluorescence. Cardiac and hepatic glycogen storage was examined. Furthermore, human cardiomyocytes were exposed to a defined trauma-cocktail and the expression levels of glucose- and fatty acid transporters were determined. Early after polytrauma, hyperglycaemia was observed. After 48 h and 72 h, pigs with fracture- and polytrauma developed hypoglycaemia. The propofol demand significantly increased post trauma. The hepatic glycogen concentration was reduced 72 h after trauma. Cardiac glucose and fatty acid transporters changed in both trauma models in vivo as well as in vitro in human cardiomyocytes in presence of proinflammatory mediators. CONCLUSIONS Monotrauma as well as polytrauma changed the cardiac energy transport by altering the expression of glucose and fatty acid transporters. In vitro data suggest that human cardiomyocytes shift to a state alike myocardial hibernation preferring glucose as primary energy source in order to maintain cardiac function.

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Cardiac glucose and fatty acid transport after experimental mono- and polytrauma

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Conflicts of Interest and Source of Funding

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Running Title: Trauma induces alterations in cardiac metabolism

Abstract

Objective: The aim of this study was to define the influence of trauma on cardiac glucose and fatty acid transport. The effects were investigated *in vivo* in a porcine mono- and polytrauma model and *in vitro* in human cardiomyocytes, which were treated simultaneously with different inflammatory substances, mimicking post-traumatic inflammatory conditions.

Methods and Results: In the porcine fracture- and polytrauma model, blood glucose concentrations were measured by blood gas analysis during an observation period of 72 h. The expression of cardiac glucose and fatty acid transporters in the left ventricle was determined by RT-qPCR and immunofluorescence. Cardiac and hepatic glycogen storage was examined. Furthermore, human cardiomyocytes were exposed to a defined trauma-cocktail and the expression levels of glucose- and fatty acid transporters were determined. Early after polytrauma, hyperglycaemia was observed. After 48 h and 72 h, pigs with fracture- and polytrauma developed hypoglycaemia. The propofol demand significantly increased post trauma. The hepatic glycogen concentration was reduced 72 h after trauma. Cardiac glucose and fatty acid transporters changed in both trauma models *in vivo* as well as *in vitro* in human cardiomyocytes in presence of proinflammatory mediators.

Conclusions: Monotrauma as well as polytrauma changed the cardiac energy transport by altering the expression of glucose and fatty acid transporters. *In vitro* data suggest that human cardiomyocytes shift to a state alike myocardial hibernation preferring glucose as primary energy source in order to maintain cardiac function.

Keywords: polytrauma, cardiac fatty acid metabolism, cardiac glucose metabolism, myocardial hibernation

Introduction

After polytrauma, patients often exhibit impaired cardiac function and cardiomyopathy, which is associated with an increased mortality rate [22]. In critically ill patients nutrition is challenging because the stress-induced catabolism after injury predisposes to malnutrition. In this context catabolic hormones such as glucagon, catecholamines and cortisol are elevated, which is associated with mobilization of endogenous energy substrates (glucose, amino acids and free-fatty acids). Of note, the delivery of these substrates to the vital organs brain and heart is prioritized [20]. The posttraumatic release of proinflammatory cytokines exaggerates catabolism [44] [17], and this stress response triggers hyperglycaemia, which is associated with increased mortality [33]. During intensive care patients are further challenged by prolonged fasting periods or feeding interruption [34]. In surgical patients malnutrition is associated with delayed healing, higher rates of complications, longer hospital stay and poor outcomes [1]. Combining different single traumata changes the levels of metabolites including glycolysis, tri-carboxylic acid cycle, pentose phosphate and fatty acid pathways [46]. Furthermore, trauma-induced hepatic stress has been associated with stress metabolism, including insulin resistance, and resulting in increased hepatic glucose production but decreased glucose uptake of the skeletal muscle [2]. In regard to the heart, we have previously demonstrated that polytrauma exhibit increased cardiac glycogen concentrations, but decreased glucose transporter 4 (GLUT4) expression in pigs [23]. For proper cardiac function a very tight coupling between ATP production and myocardial contraction is essential, supposing a constant provision of metabolites necessary for ATP production [29]. The heart is capable of utilizing all classes of energy substrates. Thereby, the use of long-chain free fatty acids (LCFA) and carbohydrates is favored [19]. In well oxygenated hearts the primary energy source for cardiomyocytes is the utilization of LCFA [38]. Another important energy source is glycogen, supporting cardiac metabolism during acute cardiac workload. However, the glycogen

turnover rate is usually very low under normal conditions [13]. Nevertheless, due to its capability to change between the different metabolisms, the human heart is able to providing a constant ATP production, and thus a proper function [12, 54]. During such cardiac metabolic reprogramming the oxidation of LCFA is mostly reduced, whereas the glucose consumption increased [4]. This shift from fatty acid oxidation to glucose utilization is especially seen in patients with chronic heart failure as well as with ischemic cardiomyopathy [48]. Given that the intramuscular storage of LCFA is limited, the heart tissue is strongly dependent on systemic supply of LCFA from adipose tissue [30]. LCFA are provided either unbound as free fatty acids or linked to albumin [36]. Its uptake by heart tissue is a complex process, involving multiple steps and many barriers [5]. The rate-limiting factor is the cellular uptake and the passage across the plasma membrane [30] since the LCFA enters cardiomyocytes either inactively by diffusion or actively *via* different transport proteins [9, 56]. Of those, we focused on cardiac fatty acid transporters, including the solute carrier 27 gene family (SLC27), the heart fatty acid binding protein (HFABP) and the fatty acid translocase (FAT/CD36). The CD36 facilitates the uptake by catalyzing the accumulation and integration of protonated LCFA into the outer phospholipid bilayer [49]. HFABP promotes the uptake of LCFA into the cytoplasm of cardiomyocytes and is responsible for the delivery of LCFA from the sarcolemma through the cytoplasm to the outer membrane of mitochondria [16, 30]. Both, CD36 and HFABP accelerate LCFA-dissociation from albumin [11]. SLC27 solute carrier proteins are integral transmembrane proteins, promoting the transport of LCFA actively across the plasma membrane. The SLC27A1 and SLC27A6 are expressed ubiquitous, whereas the SLC27A6 is almost exclusively expressed in cardiac tissue [3, 47]. In cardiomyocytes the glucose transporters GLUT1 and GLUT4 are mainly involved in glucose transport [31].

Trauma is associated with whole-body inflammation, upregulating cytokines, complement factors and damage-associated molecular patterns (DAMPs) like extracellular histones [18, 21,

[39, 45]. Although it has been shown that cytokines and endogenous danger molecules may have negative effects on the heart, leading to cardiac suppression and cardiac dysfunction [25, 43], the exact influence and underlying mechanisms are still obscure [15]. Furthermore, little is known about the impact of trauma on cardiac glucose and fatty acid transport. Trauma is able to induce an imbalance between glucose and LCFA utilization in cardiomyocytes, leading finally to impaired cardiac function. Therefore, investigations regarding cardiac glucose and fatty acid transport during trauma are of huge importance [8]. The aim of this report was to define the effects of single- and polytrauma on the cardiac glucose and fatty acid transport *in vivo* as well as *in vitro*.

Materials and Methods

In vivo experiments

Animals

The animal experiments were conducted in the framework of the TREAT consortium. This study presents results from a large animal porcine polytrauma model and all pigs from this animal experiments were enclosed in the present investigations. The model has been previously described in detail by Horst et al. [17]. Animal experiments adhere to the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines for reporting animal research [28].

All procedures conformed to the Society of Laboratory Animal Science (GV-SOLAS) as well as the National Animal Welfare Law and were approved by the responsible governmental authorities ("Landesamt für Natur, Umwelt und Verbraucherschutz": LANUV-NRW, Germany: AZ TV-No.: 84-02.04.2014.A265). This study was performed according to the guidelines of the Federation of European Laboratory Animal Science Association (FELASA).

For the investigations, n=30 12-16 weeks old male pigs with a mean of 30 ± 5 kg body weight (*Sus scrofa dormestica*, Tierzucht GmbH Heinrich, Heinsberg, Germany) were included in the study.

Anaesthesia

For premedication, pigs received an intramuscular injection with azaperone (3-4 mg/kg body weight. Anaesthesia was performed by intravenous application of propofol (2,6-diisopropylphenol) and sufentanil. Anaesthesia was maintained during the study period with propofol (1-2 mg/kg body weight). Pain medication was ensured by sufentanil (0.05 mg/ml) perfusion over the whole observation period. Vital parameters were monitored on a regular basis by electrocardiographic (ECG), pulse oximetry and blood gas analysis. Data on vital signs (heart frequency, mean arterial pressure, pO_2 and FiO_2) were checked according to the time points of whole blood sampling (1.5, 3.5, 5.5, 24, 48 and 72 h after trauma). Maintaining of body temperature was performed by using a forced-air warming system. Pigs were bedded every 6 h in order to prevent positional damage and organ compromise. Lung function was maintained by lung-protective ventilation and changing position. Liver function was monitored by aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) measurements. Kidney function was controlled by sampling of urine and measuring systemic creatinine values.

Fluids were administered by continuous crystalloid infusion (Sterofundin ISO®; 2 ml/kg body weight /h). The pigs were then orotracheally intubated and ventilation was conducted in biphasic positive airway pressure (BiPAP) mode [17]. Detailed information about animal care after trauma were described by Horst et al. [17].

Nutrition

Pigs received 16.6 ml/kg/24 h Amminopäd® 10% (Baxter, Unterschleißheim, Germany), except on the day of trauma.

Monotrauma Pig (Fracture)

Pigs underwent either monotrauma with femur fracture (n=12) or sham procedure (n=6). The sham procedure included solely anaesthesia without trauma. The monotrauma fracture group (n=12) was randomized into two therapeutic groups (n=6). One group received an external fixation of the femur fracture corresponding to damage control orthopaedics (DCO), the other group were treated with femoral nailing in accordance to early total care principles (ETC).

Polytrauma Pig

The applied polytrauma porcine model was previously published [17]. In brief, pigs with polytrauma (n=12) underwent a combination of blunt chest trauma, penetrating liver injury and femur fracture, followed by pressure-controlled hemorrhage (mean arterial pressure of 40 ± 5 mm Hg, maximal withdrawal of 45% of calculated total blood volume) for 90 min. The polytrauma group (n=12) was randomized in two therapeutic arms (n=6); external fixation of the femur fracture corresponding to damage control orthopaedics (DCO) or femoral nailing reflecting early total care (ETC).

Sample collection

Full blood samples were collected prior to trauma, directly as well as 1.5, 3.5, 5.5, 24, 48 and 72 h after trauma. After centrifugation (2000 g, 15 min, 4°C), serum was removed and stored at -80°C until analysis. Heart tissue from left ventricle as well as liver tissue were collected 72 h after trauma and were either fixed with 4% formalin followed by embedding in paraffin or quick-frozen in liquid nitrogen followed by storage at -80°C.

***In vitro* experiments**

Human stem cell-derived cardiomyocytes (iPS) from Cellular Dynamics (Madison, WI, USA) were cultured in iCell Maintenance Medium for 10 days at 37°C in an atmosphere of 7% CO_2. Cells were then treated with polytrauma cocktail (PTC, Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/SHK/A904), supplemented with 20 µg/ml whole histone fraction from calf thymus (Sigma Aldrich, St. Louis, MO, USA) for 6 h at 37°C in an atmosphere of 7% CO_2. The control group was cultured in iCell Maintenance Medium. The concentrations of the respective cytokines included in the PTC were adjusted to concentrations measured in the blood of polytrauma patients. Concentrations of extracellular histones were adjusted to serum levels in the pigs (13).

RNA Isolation

RNA from left ventricles was isolated using TRIzol™ reagent (Invitrogen, Carlsbad, CA, USA). Remaining DNA was digested using DNaseI Amplification Grade Kit (Invitrogen, Carlsbad, CA, USA). For RNA isolation from cell lysates, ISOLATE II RNA Mini Kit (Meridian Bioscience, Cincinnati, OH, USA) was used and remaining DNA was digested by DNase I (Meridian Bioscience, Cincinnati, OH, USA).

Quantitative reverse transcribed polymerase chain reaction (RT-qPCR)

The respective RNA samples were reverse transcribed in cDNA using SuperScript™ IV VILO™ MasterMix (Invitrogen, Carlsbad, CA, USA). For quantitative PCR the PowerUp™ SYBR™ Green Master Mix was used (Applied Biosystems, Waltham, MA, USA). All procedures were performed according to the manufactures instructions. For qPCR the QuantStudio3 system (Applied Biosystems, Waltham, MA, USA) was utilized. Following genes were examined from pig and from human, respectively: GLUT1, GLUT4, SLC27A1,

SLC27A6, FABP3 and CD36 (Supplemental Table 3, Supplemental Digital Content 2, http://links.lww.com/SHK/A905 and Supplemental Table 4, Supplemental Digital Content 3, http://links.lww.com/SHK/A906). mRNA expression of the respective genes was normalized to GAPDH expression. Quantitative mRNA expression was calculated by the cycle threshold method ΔΔCt. Results are presented as mean fold change.

Immunofluorescence staining (IF) and PAS staining

Paraffin sections of the respective left ventricles were deparaffinized and rehydrated. Antigen unmasking was performed in 10 mmol/L citrate buffer (pH 6) at 100°C and unspecific binding sites were blocked with 5% goat serum. Specific antigen binding was performed by incubation with the respective first antibody (Supplemental Table 2, Supplemental Digital Content 4, http://links.lww.com/SHK/A907). Specific antibody binding was detected with AlexaFluor®488-labeled second antibody (Jackson Immunoresearch, West Grove, PA, USA). Nuclei were counterstained with Hoechst and sections were mounted. Expression of the respective receptors was analyzed by fluorescence microscopy using an Axio ImagerM.1 microscope and the Zeiss AxioVision software 4.9 (Zeiss, Jena, Germany) with 40x magnification (N.A. 0.75). Fluorescence intensities were evaluated using ImageJ software (National Institutes of Health, Bethesda, MD, USA) and results are presented as mean pixel density of each group.

PAS staining was performed using PAS-staining-Kit (Merckmillipore, Darmstadt, Germany). Signal density was measured using an Axio ImagerM.1 microscope and the Zeiss AxioVision software 4.9 (Zeiss, Jena, Germany) with 40x magnification (N.A. 0.75). Results are presented as mean density of each group (arbitrary units).

Statistical Analysis

A GraphPad Prism 7.0 software was used for statistical analysis (GraphPad Software, Incorporated, San Diego, Ca, USA). All values are expressed as means ± SEM. Data were analyzed by one-way ANOVA followed by Dunnett's or Tukey's multiple comparison test. $p<0.05$ was considered statistically significant.

Results

Blood glucose and propofol concentrations during polytrauma, fracture and sham procedure

In order to determine the blood glucose concentrations during trauma, blood gas parameters were measured before, directly as well as 1.5, 3.5, 5.5, 24, 48 and 72 h after trauma. Pigs subjected to polytrauma exhibited hyperglycaemia immediately after trauma in both treatment groups (fig. 1 A). In polytrauma- and in fracture groups, blood glucose levels significantly decreased between 48 h and 72 h in the respective treatment groups compared to their baseline levels, developing hypoglycaemia (fig. 1 A). Blood glucose levels from pigs subjected to fracture increased during the first 5 h, but decreased 24 h thereafter (fig. 1 A). Sham treated animals likewise developed hypoglycaemia after 48 h. Propofol demand increased significantly in all groups after 48 h and 72 h compared to baseline levels (fig. 1 B).

Cardiac and hepatic glycogen amount after polytrauma, fracture and sham procedure

Tissue from heart as well as from liver was collected 72 h after polytrauma and after fracture, followed by DCO or ETC treatment or after sham-procedure, respectively. Additionally, liver tissue from non-treated healthy pigs of a historical group was collected and used as control. The hepatic glycogen amounts of sham-treated, fracture and polytrauma pigs decreased compared to healthy control pigs (fig. 2 A, B). Further, the hepatic glycogen amount of fracture pigs treated with ETC and of polytrauma pigs, treated with DCO decreased

significantly after 72 h compared to the control group (fig. 2 A, B). Cardiac glycogen storage decreased significantly after 72 h in the fracture group with ETC treatment, compared to the control group (fig. 2 C, D).

Cardiac glucose transport in pigs after fracture

Tissue of left ventricles was collected 72 h after fracture, ensued by DCO or ETC treatment or after sham procedure, respectively. GLUT1 mRNA expression significantly decreased in both, DCO and ETC treated pigs, whereas the GLUT1 protein levels did not differ considerably between the respective treated groups (fig. 3 A, B). GLUT4 mRNA expression significantly increased in pigs treated with ETC (fig. 3 C).

Cardiac fatty acid transport in pigs after fracture

The SLC27A1 mRNA expression did not differ between the respective treated groups, whereas the SLC27A6 mRNA expression decreased significantly in pigs treated with DCO (fig. 4 A, B). The HFABP mRNA expression decreased significantly in both treated groups, whereas on protein level no differences were visible between the respective groups (fig. 4 D, E). The CD36 mRNA expression and the CD36 protein expression decreased significantly in animals treated with DCO (fig. 4 F, G, Supplement fig.1 A, Supplemental Digital Content 5, http://links.lww.com/SHK/A908).

Cardiac glucose transport in pigs after polytrauma

Tissue homogenates and sections of left ventricles were collected 72 h after polytrauma, followed by DCO or ETC treatment or after sham procedure, respectively. GLUT1 mRNA levels decreased significantly in pigs obtained ETC treatment compared to pigs received sham procedure or DCO treatment (fig. 5 A). On protein level, the extracellular GLUT1 expression did not differ significantly between the groups (fig. 5 B, Supplement fig. 1 B,

Supplemental Digital Content 5, http://links.lww.com/SHK/A908), whereas the intracellular GLUT1 protein expression increased significantly in ventricles from pigs subjected to DCO treatment (fig. 5 C, Supplement fig. 1 C, Supplemental Digital Content 5, http://links.lww.com/SHK/A908).

Cardiac fatty acid transport in pigs after polytrauma

SLC27A1 mRNA expression increased significantly in pigs with DCO treatment compared to pigs with sham procedure. SLC27A1 mRNA expression decreased significantly in ETC treated pigs compared to pigs with DCO treatment (fig. 6 A). SLC27A6 mRNA expression increased significantly in pigs that received DCO or ETC treatment, whereas the extracellular SLC27A6 protein expression decreased in both treated groups (fig. 6 B, C, Supplement fig. 1 D, Supplemental Digital Content 5, http://links.lww.com/SHK/A908). HFABP mRNA expression and protein expression increased significantly in pigs obtained DCO treatment (fig. 6 D, E). CD36 mRNA expression decreased significantly in pigs with ETC treatment, whereas the protein expression level of CD36 did not differ significantly between the respective groups (fig. 6 F, G, Supplement fig. 1 E, Supplemental Digital Content 5, http://links.lww.com/SHK/A908).

Cardiac glucose and fatty acid transport in human cardiomyocytes in presence of polytraumacocktail and extracellular histones

Human cardiomyocytes were incubated for 6 h with PTC and additional extracellular histones. The control group was incubated in cell culture medium. GLUT1 mRNA expression increased significantly in cardiomyocytes treated with PTC and extracellular histones, whereas the GLUT4 mRNA expression did not differ significantly between the treated and the control group (fig. 7 A, B). SLC27A1 and SLC27A6 mRNA expression did not differ considerably between the respective groups (fig. 7 C, D). HFABP and CD36 mRNA

expression decreased significantly in cardiomyocytes treated with PTC and extracellular histones (fig. 7 E, F).

Discussion

In this report the effects of severe polytrauma and fracture on cardiac glucose- and fatty acid utilization were described *in vivo* and *in vitro* in presence of an inflammation cocktail. Polytrauma in pigs induced a hypermetabolic stress response, which is reflected by early increased blood glucose concentrations. This trauma-related metabolic stress response is caused by increased concentrations of circulating catecholamines and glucocorticoids, which is also a well-known problem in traumatized patients, and which is further associated with increased mortality rates as well [10, 26]. Of note, adequate pre-medication by analgo-sedation with sufentanil did not reduce high baseline glucose levels in all groups. The depleted glycogen storage in liver by the respective trauma models might also be due to increased glycolysis after trauma. Furthermore, enhanced metabolic rate (20-25%) was observed after trauma and was associated with the severity of trauma [42]. Peripheral insulin resistance during hyperglycaemia probably leads additionally to enhanced hepatic glycolysis and further decreased glucose uptake of the skeletal and cardiac muscle tissue [33]. This hyperglycaemic state is often critical for multiply injured patients, rising their risk for mortality [26, 33]. Interestingly, cardiac glycogen concentration, which has been associated with protection against trauma-induced cardiac damage [40], decreased in animals after monotrauma, but not after polytrauma as demonstrated before [23]. After initial hyperglycaemia in pigs post fracture and ETC treatment and in pigs post polytrauma, mean systemic glucose concentration dropped below 100 mg/dl. GLUT1 mRNA expression decreased in both trauma models after 72 h, indicating extensive glucose utilization during trauma. GLUT4 expression was not significantly altered between the respective trauma groups [23]. But this was probably due to the trauma-dependent insulin resistance, since insulin predominantly stimulates the GLUT4

expression of the cardiomyocytes [31]. One limitation factor for the present analysis of glucose consumption after trauma might be the small animal numbers, wherefore these results should be interpreted carefully. Therefore, more experiments are necessary to generalize the glucose consumption after trauma in pigs. However, the present data are consistent with the hypothesis of an increased glucose consumption after experimental polytrauma (13).

An increased demand of propofol in all groups during the observation period was observed. Though the propofol concentration was adapted to the depth of anaesthesia of the pigs in the present study. This may result in a metabolic switch from oxidative phosphorylation to glycolysis by targeting mitochondrial complexes I, II and III. Further, increased propofol concentration lead to disrupted β-oxidation, reduced mitochondrial entry of LCFA and consequently to accumulation of free fatty acids in cells [27]. Excess propofol concentrations are known to lead to enhanced levels of acylcarnitines and inhibit transport protein carnitine palmitoyl transferase I (CPT1) and uncouple the mitochondrial respiratory chain in humans [53]. The disturbance of mitochondrial electron transport also causes generation of free oxygen reactive species (ROS), resulting in cardiomyocyte apoptosis [32]. This phenomenon was also observed in humans, called propofol infusion syndrome (PRIS). PRIS is associated with impaired mitochondrial function and is observed in humans with propofol demands larger than 4 mg/kg per body weight and at exposure times longer than 48 h [50, 53]. In rats, propofol concentration in tissue during intravenous application (20 mg/kg body weight) reached 200 μM and was also correlated with impaired electron flow through the respiratory chain, with coenzyme Q as the main interaction site of propofol [51]. Furthermore, propofol induces contractile dysfunction in the rat myocardium [14]. The upregulation of the GLUT1 in cardiomyocytes during trauma might be protective, preventing propofol-induced apoptosis of cardiomyocytes [37]. GLUT4 expression was probably reduced by the presence of excessive lipids and free fatty acids due to increased propofol infusion [52]. Moreover, excess exposure to

saturated long chain fatty acids leads to a state of so-called cardiac lipotoxicity, including impaired cardiac glucose metabolism, impaired insulin and catecholamine signaling, cellular inflammation, excess reactive oxygen species (ROS) production, inhibition of autophagy and activation of apoptosis [6].

Since glucose consumption increased in the present trauma models in pigs with fracture or with polytrauma, cardiac utilization of LCFA was mostly reduced. The CD36 expression was completely downregulated in both trauma models. This was probably caused by a trauma-dependent insulin resistance, since the expression of the CD36 is strongly dependent on insulin stimulation [7]. Decreased CD36 expression could also be due to the profopol-induced shift from β-oxidation to glycolysis [7]. The HFABP expression decreased in pigs 72 h after fracture but in contrast increased in pigs 72 h after polytrauma, receiving DCO treatment. The increased HFABP expression in polytrauma animals indicates some post-production of the protein most likely compensatory to loss of HFABP early after polytrauma [23]. SLC27A1 and SLC27A6 expression also increased in pigs with polytrauma but not in pigs with fracture. The enhanced expression of the SLC27A1, SLC27A6 as well as of HFABP in pigs with polytrauma might be due to the increased demand for LCFA due to the fast glucose release and consumption directly after trauma. Since the upregulation of SCL27A1, SLC27A6 and HFABP was only observed in pigs with polytrauma, we suggest that the effects were not due to increased circulating propofol concentration but correlate with the severity of trauma.

Summarized, the posttraumatic environment *in vivo*, influenced by inflammation, DAMPs, intensive care nutrition and anaesthesia changed cardiac substrate utilization by adjusting different transporters, which are involved in cardiac fatty acid- and glucose transport. In mono- and polytrauma, cardiomyocytes modified their substrate utilization from LCFA to glucose, indicating for high energy requirement of the heart during trauma. However, after

polytrauma, expression of cardiac LCFA transporters increased, indicating for trauma-dependent but propofol-independent cardiac LCFA utilization, due to increased energy requirements and inflammation, correlating with the severity of trauma. Moreover, the observed alterations in the energy metabolism of the multiple injured pigs might cause their disturbed and impaired cardiac function after trauma [23, 24, 41, 55]. In order to get a closer understanding about the molecular mechanisms, leading to a cardiac metabolic switch after trauma *in vivo*, additional observation points before 72 h are required.

In vitro, changes of glucose and LCFA transporters in human cardiomyocytes in presence of an inflammation cocktail confirmed the *in vivo* observed changes. As *in vivo*, the GLUT1 expression increased, whereas the GLUT4 expression was not influenced by trauma, assuming increased glucose transport via the GLUT1 [37].

It is tempting to speculate that the increased expression of the GLUT1 was due to myocardial hibernation, which includes the downregulation of oxygen consumption and the shift to anaerobic glycolysis for cardiac ATP production [35]. Due to this adaptive response, cardiomyocytes are able to maintain their viability in state of energy deficiency. The responsible enzyme for the induction of the hibernation is the cytochrome oxidase of the electron transport chain in mitochondria [35]. In human cardiomyocytes, the expression of all transporters involved in cardiac fatty acid transport was reduced, confirming the shift to preferred glucose utilization of cardiomyocytes during treatment with DAMPs as well as with inflammatory mediators [35, 37].

In conclusion, the data indicate that human cardiomyocytes skip to the condition of myocardial hibernation, when treated with PTC and extracellular histones, utilizing glucose as primary and preferred energy source in order to maintain cardiac function and contractility of cardiomyocytes.

Authors' contributions: Regarding contributions of the authors I.L., B.W. and D.K performed the experiments including animal studies, cell culture experiments, microscopic studies and ELISAs. I.L. primarily wrote the paper K.H., B.R., F.G., H.C.P., M.H.L, F.H. and M.K. contributed to experimental design and data analysis and coordinated the study and supervised financial support for the studies. All authors made substantial contributions to conception and design of the study and participated in drafting the article. All authors gave final approval of the version to be published.

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Figure Legends

Figure 1: Blood glucose (mg/dl) (A) and propofol (ml/h) (B) values from blood gas analysis (BGA) in pigs with polytrauma, fracture or sham procedure. Pigs received either polytrauma or fracture, followed either by damage control orthopaedics (DCO) treatment or by early total care (ETC) treatment. Control pigs received sham procedure. Time of measurement was directly after trauma (baseline) and 1.5, 3.5, 5.5, 24, 48 and 72 h after trauma. n=6 pigs in each group. *p<0.05 differences to baseline concentrations.

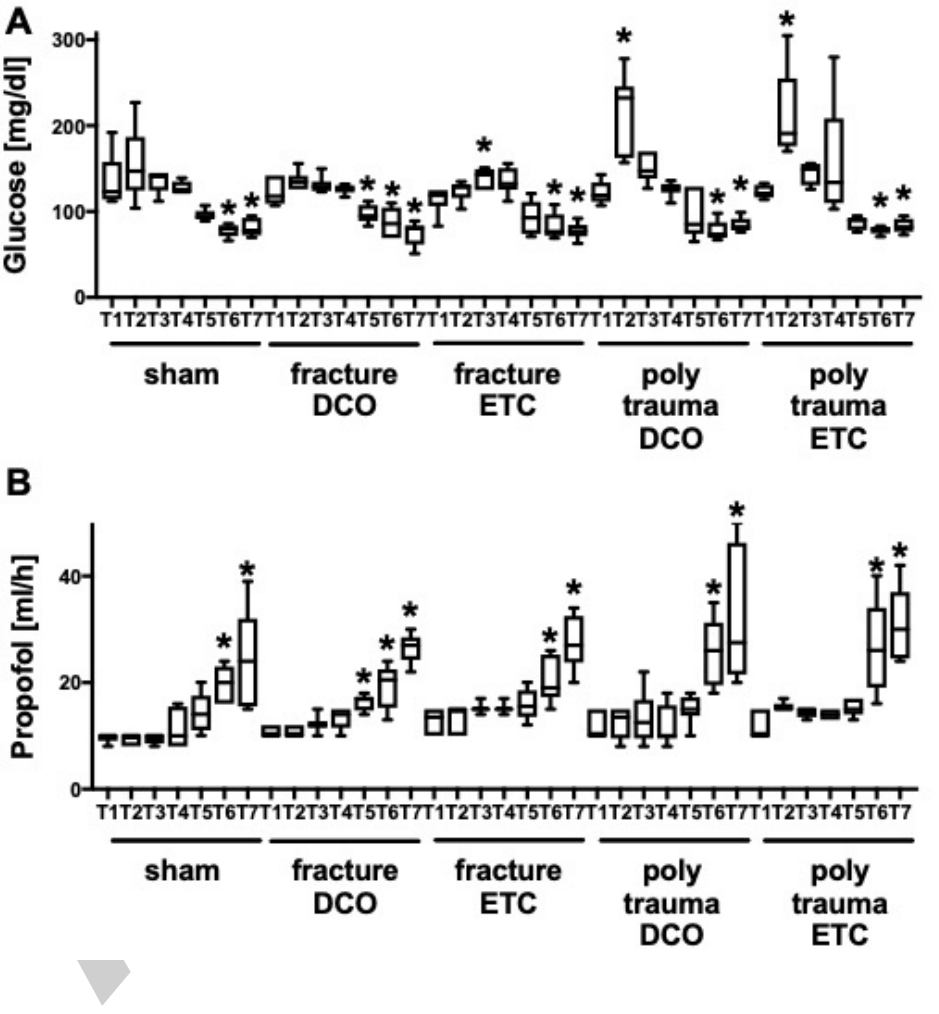

Figure 2: Hepatic (A, B) and cardiac (C, D) glycogen amount (pixel density) of sham-treated, fracture- and polytrauma and control pigs. Pigs either underwent fracture or polytrauma, followed either by damage control orthopaedics (DCO) treatment or by early total care (ETC) treatment. Control animals received sham procedure. Further, liver tissue from non-treated healthy pigs from historical group was collected (control). Liver tissue was collected 72 h after trauma. n=6 pigs in each group. *p<0.05.

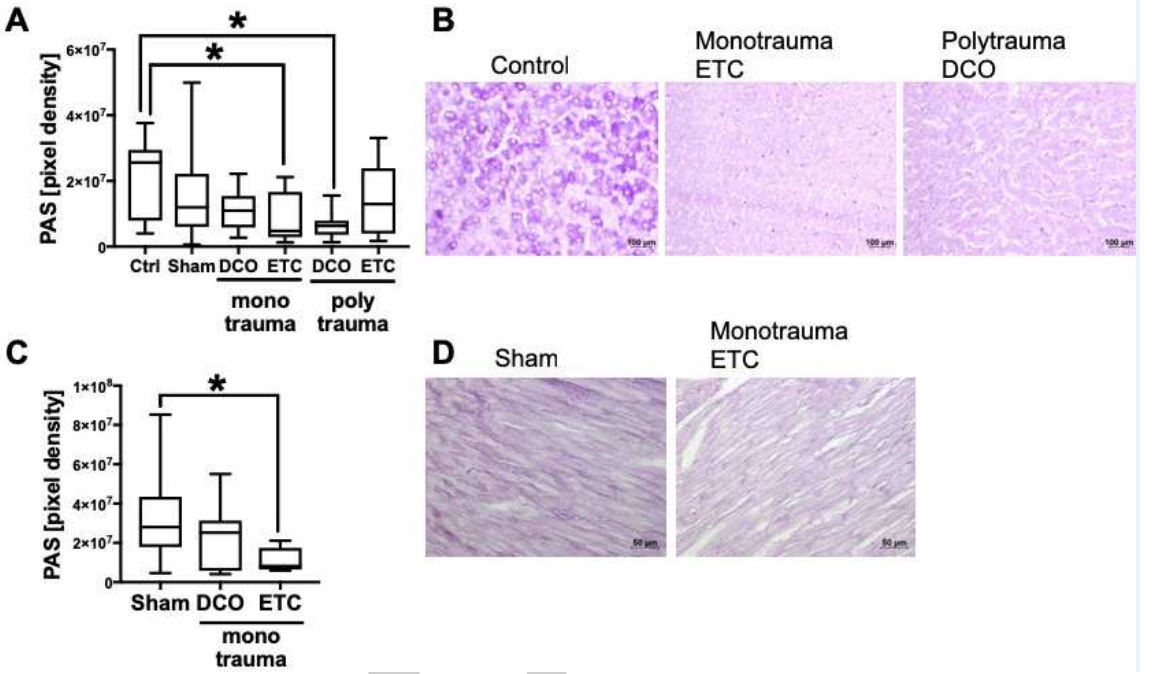

Figure 3: Cardiac glucose transport in pigs with fracture. The fold change of glucose 1 transporter (GLUT1) (A) and glucose 4 transporter (GLUT4) (C) mRNA, as well as the pixel density of the extracellular GLUT1 protein expression (B) is demonstrated. Pigs received fracture, followed either by damage control orthopaedics (DCO) treatment or by early total care orthopaedics (ETC) treatment. Control group received sham-procedure. Left ventricles were collected 72 h after trauma. n=6 pigs in each group. *p<0.05.

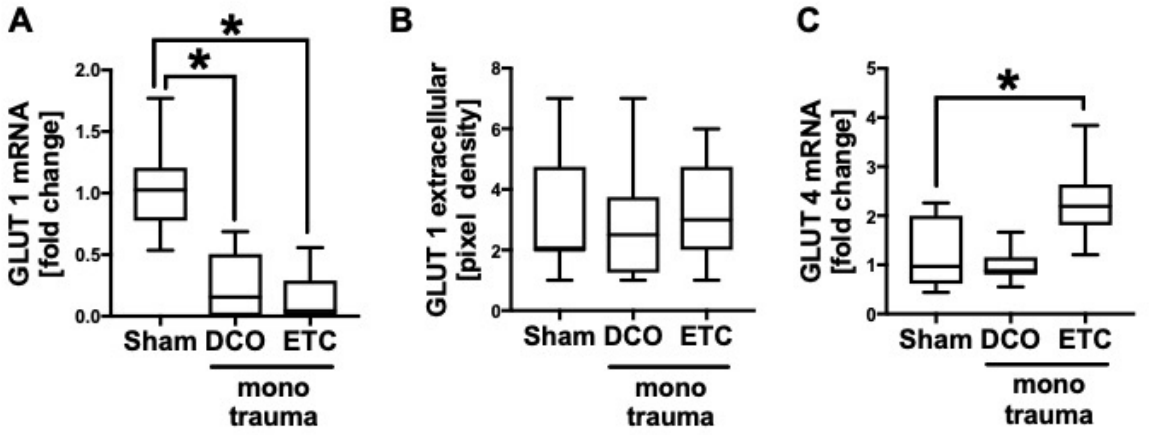

Figure 4: Cardiac fatty acid transport in pigs with fracture. The expressions of fatty acid transporter 1 (SLC27A1) (A), fatty acid transporter 6 (SLC27A6) (B), heart fatty acid binding protein (HFABP) (D) and fatty acid translocase (CD36) (F) mRNA, as well as the pixel density of SLC27A6 (C), HFABP (E) and CD36 (G) protein expression is demonstrated. Pigs received fracture, followed either by damage control orthopaedics (DCO) treatment or by early total care (ETC) treatment. Control groups were sham treated. Left ventricles were collected 72 h after trauma. n=6 pigs in each group. *p<0.05.

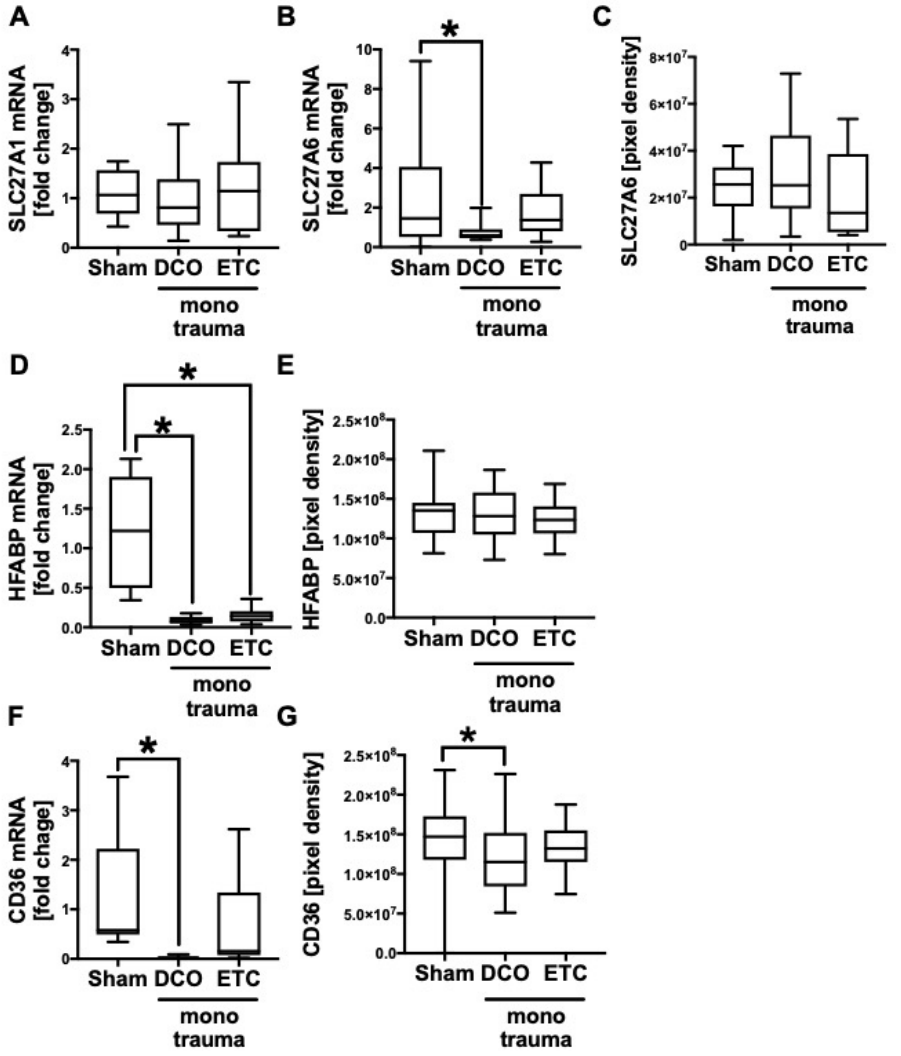

Figure 5: Cardiac glucose transport in pigs with polytrauma. The fold change of Glucose 1 transporter (GLUT1) (A) mRNA, as well as the pixel density of extracellular (B) and intracellular GLUT1 (C) protein expression is demonstrated. Pigs received polytrauma, followed either by damage control orthopaedics (DCO) treatment or by early total care (ETC) treatment. Control group was undergoing sham procedure. Left ventricles were collected 72 h after trauma. n=6 pigs in each group. *p<0.05.

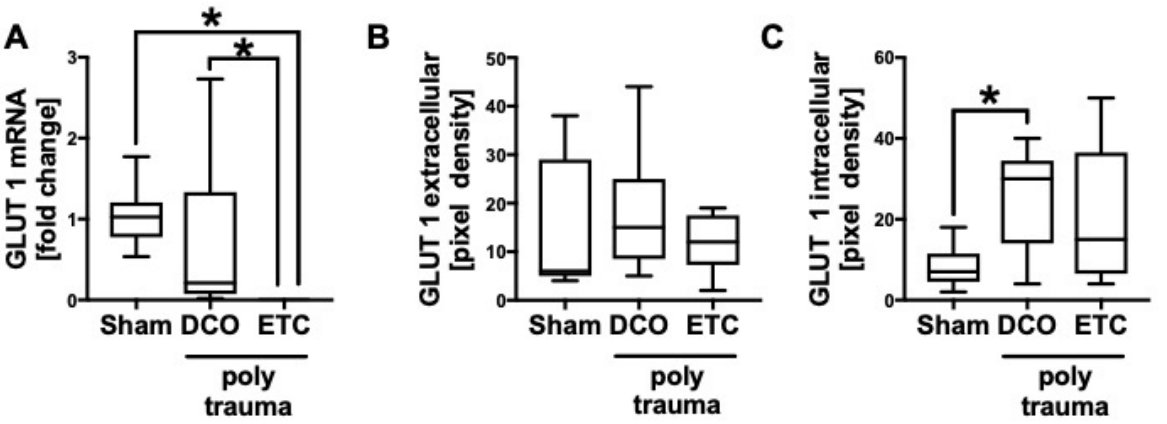

Figure 6: Cardiac fatty acid transport in pigs with polytrauma. The mRNA expression of fatty acid transporter 1 (SLC27A1) (A), fatty acid transporter 6 (SLC27A6) (B), heart fatty acid binding protein (HFABP) (D) and fatty acid translocase (CD36) (G) mRNA as well as the pixel density of extracellular SLC27A6 (C), HFABP (E) and CD36 (G) protein expression is demonstrated. Pigs received polytrauma, followed either by damage control orthopaedics (DCO) treatment or by early total care (ETC) treatment. Control group was undergoing sham procedure. Left ventricles were collected 72 h after trauma. n=6 pigs in each group. *p<0.05.

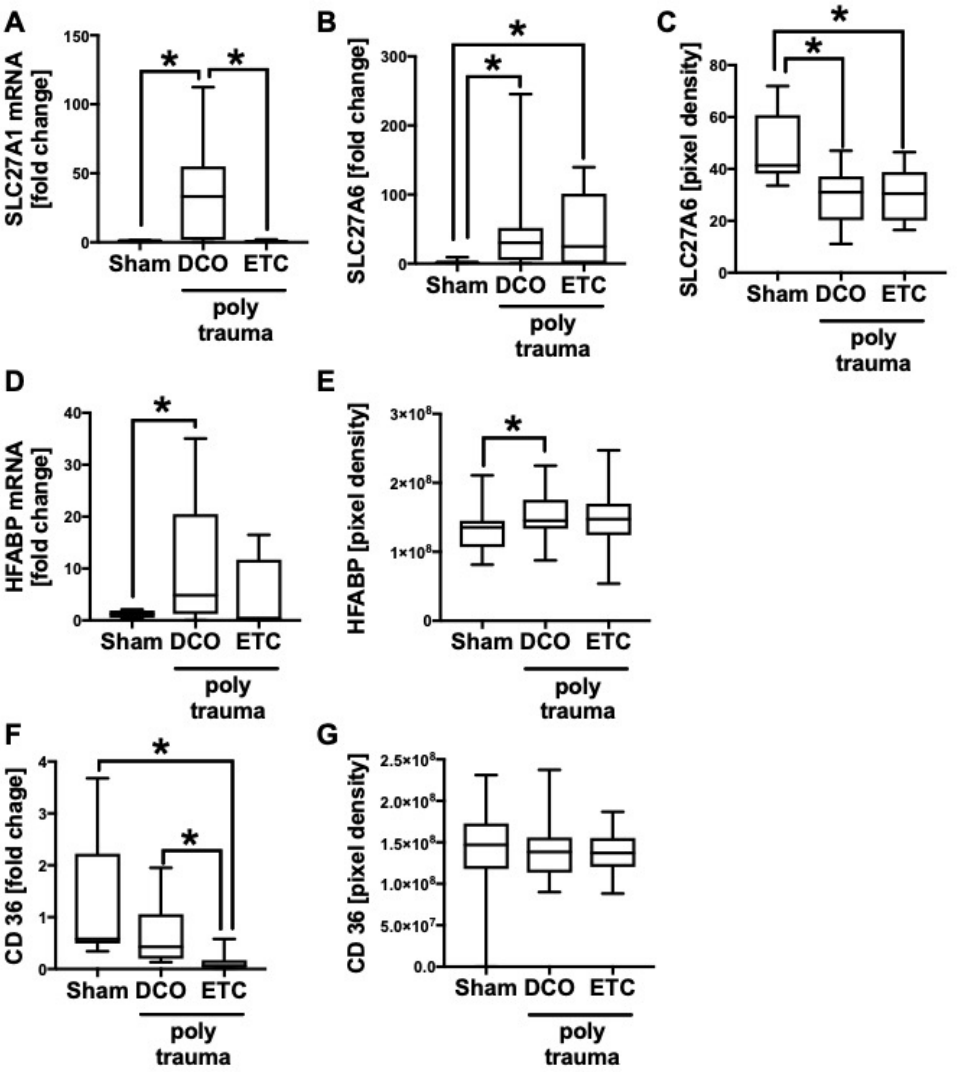

Figure 7: Cardiac glucose- and fatty acid transport in human cardiomyocytes. Human cardiomyocytes were either sham-treated or incubated for 6 h with polytraumacocktail PTC (C3a 500 ng/ml; C5a 10 ng/ml; IL-1β 200 pg/ml; IL-6 500 pg/ml; IL-8 150 pg/ml; TNFα 10 ng/ml) and 20 µg/ml extracellular whole histone fraction mix. The mRNA expression of glucose transporter 1 (GLUT1) (A), glucose transporter 4 (GLUT4) (B), fatty acid transporter 1 (SLC27A1) (C), fatty acid transporter 6 (SLC27A6) (D), heart fatty acid binding protein (HFABP) (E) and fatty acid translocase (CD36) (F) mRNA is illustrated. n=6 in each group. *p<0.05.